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## Original Research Article

# Assessment of the pregnancy rates using sequential day 3 and day 5 embryo transfer in IVF/ ICSI patients

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### ABSTRACT

**Background:** To assess the pregnancy rates using sequential day 3 and day 5 embryo transfer in IVF/ ICSI patients.

**Methods:** This prospective study was conducted in Aarogya Hospital and test tube baby Centre, Raipur from 1<sup>st</sup> January 2013 to 30<sup>th</sup> November 2019. Total 100 patients undergoing IVF/ICSI in the study period were offered sequential transfer.

**Results:** Our fertilization rates were 80% with 85.7% grade I embryos on day 3. Blastocyst formation rate was 71.42%. Cycle cancellation rates were nil. Clinical pregnancy rates per retrieval cycle were 50% and implantation rates were 24% with acceptable multiple pregnancy rates of 12%.

**Conclusions:** We advocate that this technique is useful in all patients having good quality embryos in adequate number for double transfer as this optimizes the chance of selection of the most viable embryo for transfer which is probably the key for a successful IVF program.

**Keywords:** Blastocyst transfer, Embryo transfer, Repeated implantation failure, Sequential embryo transfer

### INTRODUCTION

The clinical pregnancy rate following IVF is usually 30-50% when there are no adverse maternal factors involving uterus, endometrium or immunological system, embryos are of good quality and adequate in number and maternal and paternal karyotypes are normal. The process of implantation involves two main components, a healthy embryo that should have the potential to implant and a receptive endometrium that should enable implantation. The "Cross-talk" between the embryo and the endometrium that finally leads to apposition, attachment and invasion of embryos is mandatory for successful implantation and subsequent normal placentation.<sup>1</sup>

The embryos obtained after IVF or ICSI are transferred into the uterine cavity either on day 2 or 3 (cleavage stage transfer) or day 5 or 6 (blastocyst transfer). Blastocyst transfer has inherent advantages compared to cleavage

stage transfer such as better synchrony with the endometrium, lesser uterine contractility, better embryo ploidy status and higher implantation potential per embryo. Inadequate uterine receptivity is responsible for approximately two thirds of implantation failures, whereas the embryo itself is responsible for one third of these failures. In human's sequential embryo transfer may be used to increase endometrial receptivity and to have all advantages of blastocyst transfer.

Sequential transfer prevents the patient and the clinician of the major disadvantage in 'pure' blastocyst transfer of the cycle cancellation due to embryonic block in which there is a failure of embryos to proceed to blastocyst stage.

The objective of the study was to assess the pregnancy rates using sequential day 3 and day 5 embryo transfer in IVF/ ICSI patients.

## METHODS

This prospective study was conducted in Department of Obstetrics and Gynecology, Aarogya Hospital and test tube baby Centre from 1st January 2013 to 30th November 2019. Total 100 patients undergoing IVF/ICSI were offered sequential transfer. Our inclusion criteria for the female partner was age <37 years, day 3 FSH level <10 IU/L, E2 <80 pg/ml, hysteroscopically normal endometrial cavity, all 10 follicles >14 µm in diameter on the day of receiving β - HCG and 5 or more cleaved embryos on day 3 post fertilization. Thorough counseling of all the patients was done and informed consent was taken.

### Ovarian stimulation

The standard gonadotrophin releasing hormone agonist long protocol (Mid luteal phase) was utilized. 0.5 ml or 1 mg of leuprolide acetate was administered for down regulation from day 21 of preceding cycle and 100-300 IU of recombinant FSH was administered daily for ovarian stimulation. Follicle growth monitoring included serum estradiol, progesterone and LH measurements and transvaginal ultrasound. When one follicle reached a diameter ≥ 18 mm or two or more follicles reached ≥17 mm and at least 10 follicles were more than 14 mm, 250 µg of recombinant human chorionic gonadotrophin was administered and oocytes were retrieved 35-36 hours later. Routine IVF or ICSI was performed 4 hours after oocyte retrieval and the oocytes were checked for fertilization 16-18 hours later. Normal fertilization was indicated by appearance of two pronuclei. Embryos were cultured in commercial sequential IVF medium. Embryos were again observed at 48 hours (day 2) and 72 hours (day 3) after oocyte retrieval. The grading criteria for the embryos were as follows: Grade I, the size of the blastomeres was uniform, with no DNA fragmentation; Grade II, the blastomere size was slightly uneven with <20% DNA fragmentation; Grade III, the blastomere size was heterogeneous or with 20-50% DNA fragmentation and Grade IV, >50% DNA fragmentation. The number and grade of the embryonic blastomeres were recorded. Good quality embryos were defined as embryos containing ≥4 cells on day 2 and ≥6 cells on day 3 with grade I-II.

In all the patients on day 3, two good quality embryos were transferred. Remaining embryos were placed in blastocyst culture medium and cultured until day 5. On day 5, one good quality blastocyst was transferred and the remaining blastocysts were frozen on the same day using vitrification technique. Blastocyst grading was done according to the Gardner and Schoolcraft grading system.<sup>2</sup> No embryo underwent assisted hatching before transfer. Luteal support consisted of 800 µg of micronized progesterone orally initiated on the day of oocyte retrieval and continued until the day of pregnancy testing. Estrogen supplementation was given in the form of oral estradiol valerate 2 mg tablets thrice a day.

### Outcome measures

The primary outcome measures were the clinical pregnancy rate and implantation rate in the fresh cycle only. The secondary outcome measure was the miscarriage rate. Pregnancy testing was performed 14 days after embryo transfer. Ultrasound examination was performed at week 7 (about 5 weeks after transfer) to assess the fetal sac number and the fetal heartbeat. Clinical pregnancy was defined as the presents of a fetal heartbeat on ultrasound examination at 7 weeks of pregnancy. The implantation rate was defined as the number of gestational sacs seen on the ultrasound divided by the total number of embryos / blastocysts transferred. Implantation rate was calculated for all patients having ET and not just those who became pregnant. Spontaneous miscarriage was defined as a clinical pregnancy loss before 20 weeks of gestation age. Multiple pregnancies were defined as two or more gestational sacs observed on ultrasound. Frozen cycles were not taken into consideration and therefore cumulative pregnancy rates are not considered in this study.

The SPSS version 22.0 software program was used for statistical analysis. No ethical approval was needed for this study.

## RESULTS

Our maximum number of patients 35% were in the age group of 27-32 years with mean age of 31 years, mean BMI was 24 and 75% patients had less than 10 years of infertility (Table 1).

**Table 1: Patient profile.**

n=100		
Age (in years)	Number	Percentage
22-27	30	30
27-32	35	35
32-37	18	18
37-42	10	10
>42	7	7
<b>Mean age: 31 years</b>		
<b>BMI</b>		
20-25	70	70
25-30	30	30
>30	Nil	Nil
<b>Mean BMI:24</b>		
<b>Duration of infertility (in years)</b>		
<5	35	35
5-10	40	40
10-15	13	13
>15	12	12
<b>Mean years of infertility: 7 years</b>		
<b>Type of infertility</b>		
Primary	70	70
Secondary	30	30

Male Factors of infertility was predominant contributing to 59% cases (Table 2).

**Table 2: Causes of infertility.**

n=100		
Factor	Number	Percentage
Male factor	59	59
<b>Sperm parameters</b>		
Azoospermia	15	25.42
Oligospermia	20	33.89
Oligoasthenoatozoospermia	15	25.42
Hypospermia	05	8.47
Necrozoospermia	02	3.38
Ejaculatory dysfunction	02	3.38
<b>Female factor</b>		
Tubal factor	10	31.25
Ovarian factor	18	56.25
Uterine factor	03	9.37
Cervical factor	01	3.12
<b>Combined factor</b>	20	20
<b>Unexplained</b>	09	09

Maximum 72% patients had antral follicle count (AFC) between 8-12 and 90% patients had adequate endometrial thickness (ET) between 7-12 mm (Table 3).

**Table 3: Relevant investigations.**

n=100		
	value	Number
<b>Hormonal assays (day 2)</b>		
FSH (in ml IU/ml)	<1	6
	1-4	85
	>4	9
AMH (in ml IU/ml)	<1	10
	1-4	65
	>4	25
Serum E2 (day 5) (pg/ml)	<500	0
	500-1000	10
	1000-1500	65
	1500-2000	25
Antral follicle count (day 2)	4-8	12
	8-12	72
	>12	16
Endometrial thickness (on day of OPU)	<4 mm	10
	4-8 mm	65
	8-12 mm	25

A total 5-10 oocytes were retrieved in 65% cases (Table 4) with mean oocyte retrieval of 7 per patient, 90% oocytes were mature (M II). Our fertilization rates were 80% with 85.7% grade I embryos on day 3. Blastocyst formation rate was 71.42%. Blastocyst were formed in all the patients and a single grade I blastocyst was transferred on day 5 to all patients. Surplus blastocyst

was frozen for 25 patients. We had transferred 2 embryos on Day 3 and one blastocyst on Day 5 in all patients. Our clinical pregnancy rates per retrieval cycle were 50% but implantation rates were 24% (Table 5).

**Table 4: Status of embryos.**

n = 100		
Number of oocytes	Patients	Percentage
<b>Retrieved between</b>		
5-10	<b>65</b>	65
10-15	<b>25</b>	25
> 15	<b>10</b>	10
<b>Mean oocyte per patient = 7 (Total 700 oocytes)</b>		
M II oocytes	630/700	90
Oocytes fertilized	560/700	80%
Grade I embryos on day 3	480/560	85.71
Blastocyst formation rate	200/280	71.42
Grade I blastocyst on day 5	140/280	50.0

**Table 5: Outcome of sequential transfer.**

n=100		
	Number	Percentage
Clinical pregnancy rates (per retrieval cycle)	50	50
Implantation	72/300*	24
Multiple pregnancy	12	12
Early Pregnancy Loss	10	10
Live pregnancy rate	40	40

\*2 day 3 embryo + 1 blastocyst transferred in all patients.

Multiple pregnancy occurred in 12% cases (Twins in all cases) and live pregnancy rates were 40%.

## DISCUSSION

There are various studies suggesting improved clinical pregnancy and implantation rates in patients with repeated IVF-ET failures. In studies by Ismail Madkour WA et al in sequential transfer group they had significantly higher pregnancy rate (43.2% vs 27.4%), clinical pregnancy rate (37.8% vs 21.9%), implantation rate (17.1% vs 10.5%) and ongoing pregnancy rate (33.8% vs 19.2%) compared to conventional day 3 transfer.<sup>3</sup> We offered double or sequential ET to all our patients having  $\geq 5$  cleaved embryos on Day 3 even if it was their first IVF cycle. It is now suggested by various studies that to improve pregnancy rates endometrial receptivity should be increased. During first (day 3) transfer the embryos (2 embryos) in each patient may induce an increase in endometrial receptivity, thereby creating a better endometrial environment for the second transfer (1 blastocyst) on day 5.

Also, co-culture of early stage embryos with endometrial epithelium may increase the success rate of IVF indicating that the interaction between the embryo and

endometrium is important. Also, insertion of catheter during first transfer may induce mechanical stimulation of the endometrium and at the same time it also makes clinician aware of the technical difficulties which may occur during transfer like uterine manipulation or cervical grasping or even cervical dilatation and so these can be avoided during blastocyst transfer. Additionally, sequential transfer increases the chance of hitting the "implantation window" and thereby improving pregnancy rates. Goto et al suggested that this method may be used not only in patients with repeated failures but also in poor prognosis patients even in their first IVF-ET in order to improve pregnancy rate.<sup>4</sup>

We got twin pregnancies in 12% of our patient Dalal et al found multiple pregnancy rate of 15.3% in the sequential transfer group.<sup>5</sup> Alone blastocyst transfer on day 5 can have high cancellation rates because if no blastocyst is formed than no embryo can be transferred. Cycle cancellation rates were nil in our study. Fang et al also reported no cycle cancellations in day 3 and day 5 transfer group.<sup>6</sup>

With sequential transfer we could achieve clinical pregnancy rate of 50% which is comparable with Loutradis D et al who reported 60% clinical pregnancy rate with an additional embryo transfer on day 4.<sup>7</sup> Tehraninejad et al found that chemical and clinical pregnancy rate was similar in the sequential ET GROUP (40%) compared to the day 5 of ET group (38.3%) in patients with three repeated IVF-ET failures.<sup>8</sup>

We would like to emphasize that surplus embryos add additional cost of freezing of embryos. If sequential transfer is done patient gets the best embryos and then surplus blastocyst can be frozen. We used vitrification for surplus blastocysts as the available data supports its potential safety.<sup>9</sup>

Limitation of the study: We had limited number of case who agreed for sequential transfer considering the fact that may couples refused to enroll in the study due to the risk of multiple gestation and wanted single embryo transfer only.

## CONCLUSION

Our experience with sequential transfer has improved our clinical pregnancy rates from 30% to 50%. We have not faced the problems of cycle cancellation. All the good quality embryos are properly utilized. It is also reduced our cost of Day 3 embryo freezing. Our experience with blastocyst freezing and its outcome still needs long term follow-up. We advocate that this technique is useful in all patients having good quality embryos in adequate number

for double transfer as this optimizes the chance of selection of most viable embryo for transfer which is probably the key for a successful IVF program.

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*Ethical approval: The study was approved by the Institutional Ethics Committee*

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